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13. ABSTRACT *(Maximum 200 words)*

The new NMR instrument is currently being used to support several research projects involving studies of tissue metabolism. Work is continuing in the area of brain and liver phospholipid metabolism. The 600 MHz NMR is providing superb resolution and sensitivity for analyses of lipid components in tissue extracts. New capabilities afforded by this instrument will enable carbon-phosphorus polarization transfer experiments to aid in our studies of ether-lipid biosynthesis in brain. Such experiments will enable us to discern acyl-glycerol phospholipids from ether-linked glycerol phospholipids. These studies will begin in the near future.

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Final Technical Report

Department of Defense
Defense University Research Instrumentation Program (DURIP)
Air Force Office of Scientific Research
Award Number: F49620-02-1-0280

Principal Investigator: Nicholas V. Reo, Assoc. Prof., Dept. Biochem. & Mol. Biol., Wright State University School of Medicine

Title: Upgrade in NMR Research Instrumentation for the Magnetic Resonance Laboratory

Instrumentation Purchase, Current Status, and Accounting Information

Funds acquired from three sources (*vida infra*) were used to purchase new NMR research instrumentation for the Wright State University (WSU), School of Medicine, Magnetic Resonance Laboratory (MRL). NMR instruments and associated products were evaluated from two vendors—Bruker Biospin Corporation and Varian NMR Systems, Inc. Our primary goal was to purchase a 600 MHz NMR spectrometer for support of NMR-based metabolomics research. However, these DURIP funds, when combined with state and university monies, helped to leverage a deal that included not only a new 600 MHz instrument, but also a replacement console for our aging 360 MHz NMR spectrometer. This additional acquisition was accomplished without additional costs to the government and will further benefit DoD-related NMR research projects.

Dr. Brent Foy (Dept. Physics, WSU) and I met with sales representatives from Bruker and Varian, and visited both vendor sites for demonstrations. Test samples were prepared in my laboratory and used to evaluate instrument performance. Bids were evaluated, and in September 2002 Varian was selected as the vendor of choice. As noted above, in addition to the Varian 600 NMR system this vendor agreed to provide a Tecmag Discovery console for our 360 MHz NMR instrument.

The Varian Inova 600 NMR Spectrometer was delivered in November 2002 and installation began in December 2002. The installation was completed on March 28, 2003. The Tecmag console was installed in March 2003. There is, however, a problem with the proton decoupler unit and full acceptance of the equipment has not yet been granted. Tecmag is currently working to correct this problem. An accounting of the purchase and available funding is provided below.

Funding Sources	Funding Amounts
DoD, DURIP	$478,485
Ohio Board of Regents, Action Fund	$235,242
Wright State University	$247,831

Item Description	Costs
(1) NMR Systems Package: Varian Unity Inova 600 MHz High-Resolution NMR 3 RF channels 40 channel shims 3-axis pulsed field gradients variable temperature unit ^{1}H observe {^{13}C/X) decouple, 5 mm triple resonance probe with z-gradient X observe {^{13}C/^{1}H} decouple, 5 mm triple resonance probe with z-gradient ^{1}H observe {X) decouple, 4 mm magic angle spinning (MAS) Nano probe (40 ul volume) SunBlade 100 Workstation and software licenses Tecmag Discovery Console for 360 NMR	$945,152
(2) Freight	$1,351
(3) Startup Cryogens	$5,050
(4) Air Compressor, air dryer, and installation of air lines	$6,108
(5) Site Preparation (electrical and T1 ethernet)	$3,897
TOTAL	**$961,558**

Research Programs

Ongoing Studies

The new NMR instrument is currently being used to support several research projects involving studies of tissue metabolism. Work is continuing in the area of brain and liver phospholipid metabolism. The 600 MHz NMR is providing superb resolution and sensitivity for analyses of lipid components in tissue extracts. New capabilities afforded by this instrument will enable carbon-phosphorus polarization transfer experiments to aid in our studies of ether-lipid biosynthesis in brain. Such experiments will enable us to discern acyl-glycerol phospholipids from ether-linked glycerol phospholipids. These studies will begin in the near future.

Our Gulf War Syndrome research program (DoD-funded) is also benefiting from this new instrumentation. Currently these studies are focused on the possible synergistic effects of combined exposure to sarin nerve agent, pyridostigmine bromide, and DEET. We are now conducting high-resolution NMR analyses of rat brainstem extracts at 600 MHz. This has provided significant improvements in spectral resolution to aid in the identification of brain metabolites that are affected by chronic exposure to chemical agents and stress.

New Studies

Recently, we have initiated new studies in the area of NMR-based metabolomics research. This work is being conducted in collaboration with Dr. Nicholas DelRaso (Research Scientist, AFRL/HEST, Wright-Patterson Air Force Base) and Dr. Stephen Gustafson (Dept. Engineering, Air Force Institute of Technology). The goal of these studies is to use NMR spectroscopy and pattern recognition technologies to characterize tissue-specific toxicity based upon the metabolic profile of urine. This specific methodology is known as metabonomics.

Developmental work has begun to optimize our methods of NMR data acquisition, data reduction and analyses. We have initiated experiments to examine the ^{1}H NMR spectrum of rat urine at 600 MHz. Such an aqueous sample presents problems with dynamic range, whereby the large signal from water obscures the much smaller metabolite signals. Therefore, these experiments must employ solvent suppression methods in order obtain a useable spectrum for analysis of metabolite signals. Presently, we are conducting experiments to evaluate different solvent suppression techniques. Preliminary data have yielded spectra with reasonably good suppression such that peaks within ±0.6 ppm of the water signal can be analyzed. These experiments are currently in progress.

Additionally, we are working to establish the appropriate data processing tools. Newly written software from Varian has been obtained which is designed to "bin" the NMR spectra. This is a process whereby the spectrum is subdivided into a specified number of bins containing the signal intensity data. The numerical data is then tabulated to provide a compatible format for principle component analyses (PCA). We are currently working to implement this software on the new Varian instrument. Dr. Gustafson will then provide assistance with data reduction and statistical analyses using PCA and pattern recognition methods.

Dr. DelRaso has recently initiated a toxicity study in rats whereby our metabonomics methods will be validated. These experiments involve treatments with known liver and kidney toxins. Urine and blood samples will be collected before and at various times post-treatment. Dr. DelRaso will then provide those samples for 600 MHz NMR analyses.

Future Research Opportunities

This new high-field NMR instrument will also provide a unique opportunity to develop methods for NMR-based metabolomics. Here I plan to collaborate with Dr. John Frazier (AFRL, WPAFB) and Dr. Brent Foy (Dept. Physics, WSU) to analyze the metabolic profile of cells during toxicity testing. This research will attempt to identify as many metabolites as possible, and then combine this information with that obtained by genomics and proteomics to develop a holistic approach to cellular network analysis. This is a very ambitious study that is still in the planning stages. Our new NMR instrument is a key component for this research program.